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On the Structure of Ophidine*

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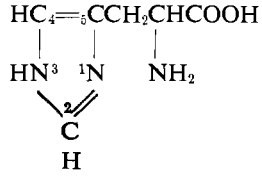
ABSTRACT: Nuclear magnetic resonance spectra and *N*-methyl analysis have shown ophidine to be β-alanyl-3-methylhistidine and not β-alanyl-2-methylhistidine.

The constitution of ophidine, obtained from snake muscle or whale tissues (Imamura, 1939; Tsunoo *et al.*, 1959; Horisaka and Musashi, 1963), was found by Kendo (1944) to correspond to β-alanyl-2-methylhistidine. The position of the methyl groups was assigned to the 2-carbon of the imidazole ring on the basis of (1) a weak Pauly reaction (Kendo, 1944; Horisaka and Musashi, 1963); (2) a hydrolysis product consistent with a methylhistidine. On pyrolysis this product yielded a methylimidazole whose picrate had a melting point like that of the 2-methyl derivative and differed from the picrate of 4-methylimidazole (Kendo, 1944); (3) R_F values of the histidyl moiety like those of 2-methylhistidine



(four solvents) (Tsunoo *et al.*, 1959); and (4) finally, the presumed structure, β-alanyl-2-methylhistidine, was synthesized by Ono and Hirohata (1956) in an unequivocal manner. The natural and synthesized compounds had identical melting points as the free base, picrate or picrolonate, as did the derived 2-methylhistidine. A mixture melting point reported only for the picrolonate (232°) showed no depression. The optical rotations of the free base and synthetic products were comparable, +41.3 and +36.6°, respectively. No infrared comparisons were reported.

During an investigation of the role *N*-iodo intermediates in the iodination of histidine, various methyl derivatives of histidine were used (Wolff and Covelli, 1967, 1968). To our surprise ophidine could not be iodinated either with I_2 or ICl despite the supposed presence of a dissociable proton on one of the imidazole nitrogens. Since carnosine was readily iodinated whereas anserine (its 1-*N*-methyl derivative) was not, a possible explanation lay in the erroneous assignment of the 2 position for the methyl group. For this reason nuclear magnetic resonance spectra of ophidine were compared with those of authentic samples of the 1-, 2-, or 3-methylhistidine.

Experimental Section

Ophidine (Table I) (mp 248°) and its picrolonate (mp 234°) were obtained from whale muscle as previously described (Horisaka and Musashi, 1963). 1- and 3-methyl-L-histidine were obtained from Mann Research Laboratories and 2-methyl-DL-histidine from Cyclo Chemical Corp. The 3-methyl derivative yielded the expected analytical values (as CH_3I) for *N*-methyl groups, whereas 2-methylhistidine yielded no *N*-methyl groups, as expected. Ascending chromatography was carried out on Whatman No. 1 or 2 paper at room temperature. Solvent compositions are listed in Table II. Nuclear magnetic resonance spectra were measured at 60 Mc in D_2O plus $NaHCO_3$ with 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt as internal standard. Infrared spectra were measured in KBr disks.

Results

Elemental analysis of the free dipeptide was consistent with the originally proposed structure of ophidine

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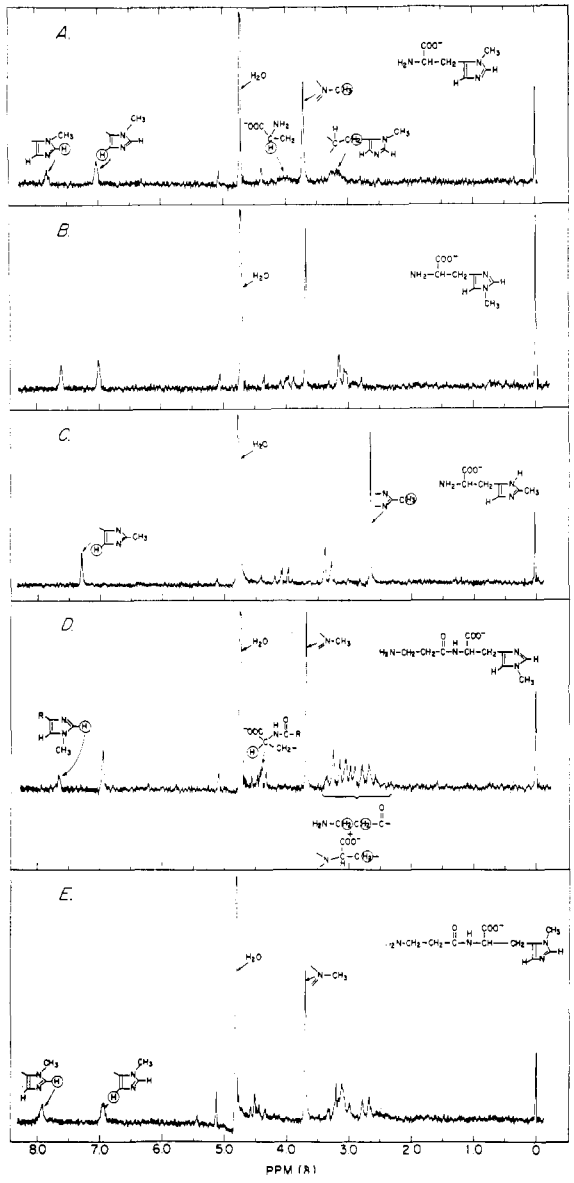

FIGURE 1: Nuclear magnetic resonance spectra of 1-, 2-, and 3-methylhistidine, anserine, and ophidine in D_2O and excess $NaHCO_3$. (A) 1-Methylhistidine, (B) 3-methylhistidine, (C) 2-methylhistidine, (D) ophidine, and (E) anserine.

but all of the methyl group could be accounted for as an *N*-methyl group by the gold chloride–HI method. Under the conditions of the *N*-methyl analysis in hydriodic acid it is unlikely that a 2-methyl group would be removed as methyl iodide. The infrared spectrum of the picrolonate was identical with those previously published (Nakai *et al.*, 1963; Tsunoo *et al.*, 1966).

Nuclear magnetic resonance spectra of ophidine were compared with spectra of authentic samples of 1-, 2-, and 3-methylhistidine as well as anserine, all run in D_2O with excess sodium bicarbonate. It is clear that ophidine and anserine possess both 2- and 4-hydrogens (δ 6.9–8.0) as do the 1- and 3-methylhistidines, in contrast to 2-methylhistidine which exhibits only one band in this region due to the 4-hydrogen atom (Figure 1).

Of the series, only 2-methylhistidine exhibits a *C*-methyl group at δ 2.67 (compare 2-methylimidazole δ 2.41). The other compounds including ophidine clearly show an *N*-methyl at $\sim\delta$ 3.69 (compare 1-methyl-imidazole δ 3.63). The product obtained after hydrolysis gave the nuclear magnetic resonance spectrum of 3-methylhistidine. Other peaks are assigned as shown in Figure 1.

TABLE I: Analysis of Free Ophidine.

	C (%)	H (%)	NCH_3 (%)
Calculated (for 2-methyl derivative)	49.99	6.71	0
Calculated (for 3-methyl derivative)	49.99	6.71	6.26
Found	49.71	6.96	6.03

Paper chromatography of the three methylhistidines reveals that the 2- and 3-methyl isomers cannot be reliably separated by the solvents previously used (solvent 1–3), nor by a new solvent that readily separates 1-methylhistidine from the other isomers (Table II).

Comment

The fact that the methyl group is entirely accounted for as an NCH_3 and the nuclear magnetic resonance comparison with the authentic 1-, 2-, and 3-methylhistidine derivatives leaves little doubt that the structure of ophidine is β-alanyl-3-methylhistidine and not β-alanyl-2-methylhistidine. Ophidine differs from anserine (β-alanyl-1-methylhistidine) in the infrared spectra, optical rotations, chromatographic behavior, and melting points of the picrate. Mixture melting points show depression (Kendo, 1944; Müller, 1959; Tsunoo *et al.*, 1966). In addition, the nuclear magnetic resonance spectrum of anserine shows the 2-proton in a position comparable with that of 1-methylhistidine, whereas the position of this proton in ophidine corresponds to 3-methylhistidine (Figure 1). The assignment of the methyl group of ophidine to the 2 position by Kendo is difficult to explain since the 1- and 2-methylimidazole picrates melt as

TABLE II: R_F Values of Methylhistidines.[a]

	1	2	3	4
1-Methylhistidine	0.09	0.52	0.09	0.14
2-Methylhistidine	0.10	0.55	0.16	0.26
3-Methylhistidine	0.13	0.54	0.12	0.24

[a] Solvents: (1) 1-butanol–acetic acid–H_2O (4:1:1), (2) pyridine–water (65:35), (3) pyridine–1-butanol–H_2O (4:1:1), and (4) pyridine–2 N acetic acid (4:1).

158–159 and 213°, respectively. Since imidazole picrate also melts at 212–213° it is possible that dealkylation occurred during pyrolysis, or alternatively, that the *N*-methyl group has migrated to the 2 position. From the above it appears highly likely that ophidine is identical with two other methylhistidine dipeptides obtained from whale muscle, cetasin (Müller, 1959), and balenine (Pocchiari *et al.*, 1962). Allowing for small differences due to sample preparation and scale representation (cm^{-1} *vs.* wavelength), the infrared spectrum of cetasin picrolonate (Müller, 1959) is apparently the same as that of ophidine (Tsunoo *et al.*, 1959). Balenine (also called isoanserine) has been synthesized from 3-methylhistidine (Rinderknecht *et al.*, 1964; Dennis and Lorkin, 1965).

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Identification of Two New β-Unsaturated Amino Acids in the Mushroom, *Bankera fuligineoalba**

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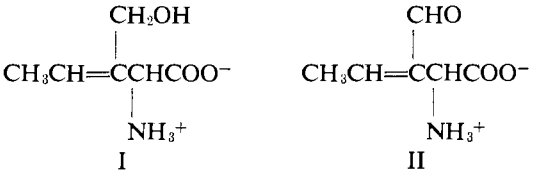
ABSTRACT: Two monounsaturated α-amino acids have been discovered in fruiting bodies of the mushroom, *Bankera fuligineoalba*. One of them, I, isolated as a pure, crystalline solid, has been characterized by chemical and spectral criteria as L-2-amino-3-hydroxymethyl-3-pentenoic acid. Nuclear magnetic resonance spectral data tentatively permit the assignment of the methyl and hydroxymethyl groups to the *cis* configuration. The other, II, was obtained in purified form, and, on the basis of its ultraviolet spectrum and its conversion into I by alkaline KBH_4 has been tentatively assigned the structure L-2-amino-3-formyl-3-pentenoic acid.

In the course of a search for new amino acids from higher fungi, we examined methanolic extracts of the mushroom, *Bankera fuligineoalba*. Using two-dimensional paper chromatographic techniques, the presence of two unusual compounds was observed, both of which afforded atypical colors after reaction on paper with ninhydrin, but only one absorbed in the ultraviolet region. The purpose of this report is to describe the isolation and properties of these compounds and to offer evidence in favor of their proposed formulation as L-2-amino-3-hydroxymethyl-3-pentenoic acid (I) and L-2-amino-3-formyl-3-pentenoic acid (II).



Experimental Work and Results

General. All concentration steps were done *in vacuo* (water aspirator) using rotary or test-tube-type flash evaporators operating at temperatures not over 35°. Infrared spectra were obtained from KBr disks using the Perkin-Elmer Model 237 spectrometer. Nuclear

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